Patent
Case No. GY20a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#9 Appl 10-23-92

Group:	1202
Examiner:	C. Shen
Applicant:	Zahler et al.
Serial No.	763,033
Filed:	September 20, 1991
For:	Hydroxymethyl(Methylenecyclopentyl)Purines And Pyrimidines

Princeton, New Jersey 08543-4000

October 9, 1992

DECLARATION UNDER 37 CFR 1.131

To the Commissioner of Patents and Trademarks:

We, Robert Zahler and William A. Slusarchyk, declare as follows:

1. That we are the inventors of United States patent application Serial No. 763,033 filed September 20, 1991 which is a continuation-in-part of Serial No. 599,568 filed October 18, 1990.

2. That the invention described in Claims 4 - 8, 15, and 23 - 26 of Serial No. 763,033 was conceived and reduced to practice by us in the United States prior to May 2, 1990.

3. That prior to May 2, 1990 the synthesis of [1S-(1α,3α,4β)]-2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one was carried out under the direct supervision of William A. Slusarchyk in the United States and reported to Robert Zahler. This compound was assigned the identification number SQ34,676. The synthetic procedure employed is shown in attachment A which is a contemporaneous document prepared by the chemist who performed the synthesis with the dates deleted.

4. That prior to May 2, 1990, samples of SQ34,676 were submitted by Robert Zahler and William A. Slusarchyk for

antiviral testing by the Virology Department of the Squibb Institute.

5. That such antiviral testing was performed in the United States and the results were reported back to Robert Zahler and William A. Slusarchyk prior to May 2, 1990. That the results of such testing are shown in Attachments B through J which are contemporaneous documents prepared by the person who conducted the tests with the date of testing deleted.

The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of application Serial No. 763,033 or any patent issued thereon.

Date Oct 9, 1992 — Robert Zahler

Date October 9, 1992 — William A. Slusarchyk

ATTACHMENT A

SQUIBB INSTITUTE
CHEMICAL TRANSMISSION RECORD
Chemistry/Infectious & Metabolic Diseases

Date	Number
	SQ-34676
Project: AVR-000	Batch: NN001

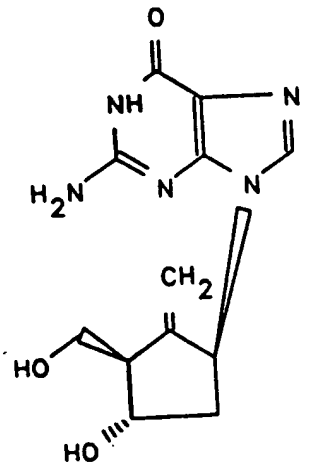
SQ-34676 [1S-(1<α,3<α,4<β)]-2-Amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one.

C12H15N5O3

MW/FW 277.28

Physical State: solid

CHIRAL



HANDLING PRECAUTIONS
Hygroscopic: yes
Stability: refrigerate
Other:

SOLUBILITY
H2O: %, pH see comments
Adjustable to pH

Novel Compound: yes

Chemist: M. G. YOUNG

Notebook: L030-195-32

Checked by: W.A. Slusarchyk

Preliminary Data Sheet:

Complete Data Sheet:

Comments: 1) 80 mg sent to Virology on
2) Not soluble in PBS buffer pH 7.2 at 5mg/ml but soluble at 0.145 mg/ml.
3) Soluble in DMSO 5 mg/ml.

Assays:

Antiviral

Elemental Analysis (%)

$C_{12}H_{15}N_5O_3 \cdot 0.9\,H_2O$

	Calc.	Found
C	49.12	49.17
H	5.77	5.87
N	23.87	23.81

m.p.: > 220°C

IR: 75180 (KBr)

1H NMR: L030195-32 ($DMSOd_6$) 270 MHz

13C NMR:

M.S.# MGY 131

U.V. λmax at pH 7.2 253.3nm (ε=12,900)

$[\alpha_D^{22°}] = +34.0$ [c=0.30, water]

Fischer:

TLC: Preliminary HI = 97.3 $CHCl_3$: CH_3OH: NH_3 (6:3:1)
HPLC:
TLE:

sent to Research Chemicals
1 mg Distribution on:
Distribution by RCD:
Microbiology:
Pharmacology:

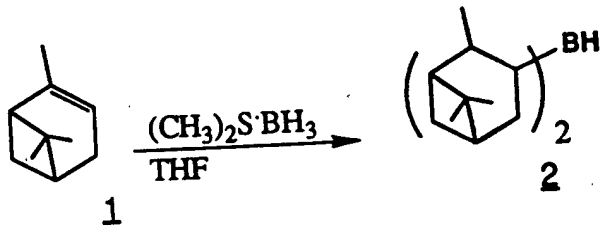
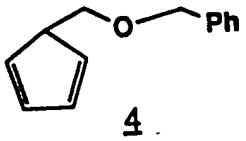
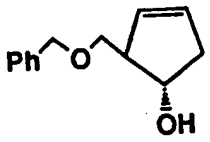
Copies to:
RIC
RCD
Dr. G. Biscocchi
Dr. C. Cimarusti
Dr. A. Field
Dr. O. Kocy
Dr. W. Koster
Dr. K. Lindner
Dr. C. Meyers
Dr. W. Scott
Dr. R. Zahler

CHEMIST'S REPORT-FLOW SHEET

OUTLINE OF PREPARATION OF:

SYNTHETIC CHEMICAL NUMBER
SQ 34,676

DATE

CHEMIST NO.	COMPOUNDS: *Start with preparation of first intermediate not previously prepared in this series. Yields should be calculated on basis of total starting material.*	NOTEBOOK PAGES	YIELDS
	Ref. 1  1 2	L030081	72%
	 3 1. Na, THF, -5° 2. Benzyl chloromethyl ether, -50°		
	Ref. 2  4 1. 2, -60° for 1 hr, -10° for 18 hr 2. 3N NaOH, 30% H_2O_2		
	Ref. 2  5 $VO(acac)_2$, t-BuOOH CH_2Cl_2	L030093	25% for 2 steps

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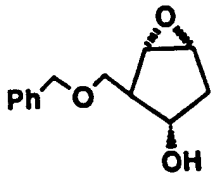
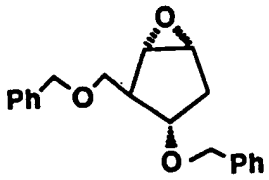
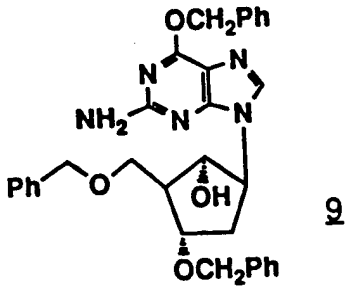
	 6	L030105	84%
	Ph CH_2Br, NaH, DMF Bu_4NI		
	 7	L030114	76%
	O-benzylguanine (8), LiH, DMF		
	 9	L030121	60%
	p-Anisylchlorodiphenylmethane, triethylamine, dimethylaminopyridine, CH_2Cl_2		

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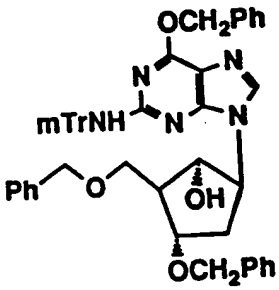
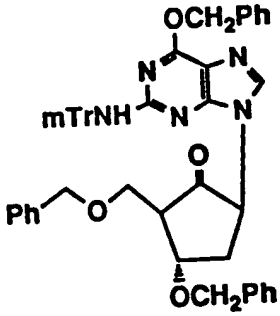
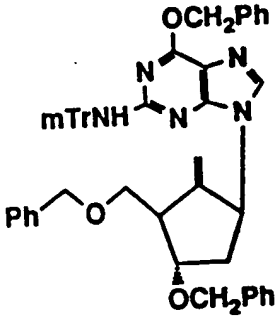
CHEMIST NO.	COMPOUNDS: *Start with preparation of first intermediate not previously prepared in this series. Yields should be calculated on basis of total starting material.*	NOTEBOOK PAGES	YIELDS
	 10	L030167	74%
	DCC, DMSO, Methyl phosphonic acid		
	 11	L030170	
	Zn, $TiCl_4$, CH_2Br_2 THF, CH_2Cl_2 Ref. 3		
	 12	L030189	

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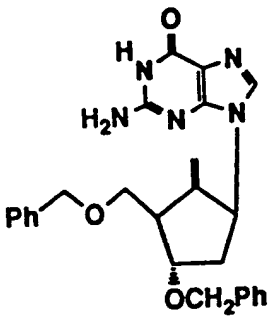
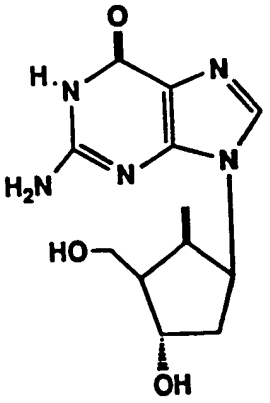
CHEMIST NO.	COMPOUNDS: *Start with preparation of first intermediate not previously prepared in this series. Yields should be calculated on basis of total starting material.*	NOTEBOOK PAGES	YIELDS
	1. aqueous 3N HCl, THF, CH_3OH 2. 1N KOH to pH 7		
	 **13**	L030190	23% for 3 steps
	BCl_3, CH_2Cl_2 -78° to -40°		
	 **14**	L030195	62%
	SQ 34,676		

References

1. H. C. Brown, et. al. JOC, 1984, 49, 945
2. K. Biggadike, et. al. J. Chem. Soc. Perkin Trans. 1988, 549

3b. L. Lombardo, Tet. Let. 1982, 23 4293

3a. S. Ahmed, Status Report June 5, 1989- November 30, 1989

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Compound 2

To a solution of 10M borane-methylsulfide (100 ml, 1mol) in THF (1 l, distilled from K) at 0° was added with stirring 1R (+)-α-pinene (158.8 ml, 1 mol) with an optical purity of less than 90%. The reaction was then placed in the cold room for 16 hr with no stirring. Then more 1R (+)-α-pinene (158.8 ml, 1 mol) was added. A precipitate began forming after 20 min and the suspension was stirred for 8 hrs at 0°. The suspension was allowed to settle for 15 min and the solvents were cannulated away. The solid was washed with ether (3x130 ml) and then dried for 16 hrs on the vacuum pump. The solid was transferred (under N_2 in a dry bag) to dry bottles and stored at -20°. Total 2 obtained was 205 g (72% yield).

Compound 3

Dicyclopentadiene (300 ml) was cracked at 186° under N_2. The cyclopentadiene was distilled through an 18 inch Vigreaux column. A total of 110.73 g (b.p. 38°) was collected and stored at -78°.

Compound 5

Cyclopentadiene (28.68 g, 0.434 mol) was warmed from -78° to -30° and cannulated to an addition funnel at -30° under N_2. This was added to 40% Na sand in oil (22.5 g, 0.391 mol) in THF (156 ml, distilled from K) over a 1 hr period keeping the temperature of the reaction at -10°. The cyclopentadienyl sodium solution was then cannulated to an addition funnel at 0° over 1.3 hr. This solution was added to benzylchloromethyl ether (65.19 ml, 0.469 mol) in THF (130 ml) at -50° over 1.3 hr.

This suspension was stirred 1.3 hr at -45° and then cooled to -60° The suspension was diluted with THF (390 ml) and then compound 2 (136 g, 0.477 mol) was added as a solid under a N_2 atmosphere. The reaction was then stirred 1 hr at -60° and warmed to -10° over 1.5 hr. This was stirred at that temperature for 16 hr. The reaction was concentrated to 1/2 the volume *in vacuo*, and the slurry was diluted with 390 ml of ether. The reaction was cooled to 0° and 3N NaOH (156 ml, 0.469 mol) was added over 45 min keeping the temperature at 0°. Then 30% H_2O_2 (156 ml) was added over 1 hr keeping the temperature below 12°. The reaction was stirred 1 hr at 10°.

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The layers were separated, and the water layer was washed with ether (300 ml). The ether layers were combined, washed with brine (200 ml), dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified on Merck silica gel (5 l packed in petroleum ether:ether, 2:1). The column was eluted with petroleum ether:ether (2:1 to 1:1) to give 20 g (25% yield) of 5.

Compound 6

To a solution of compound 5 (29.63 g, 0.145 mol) and vanadyl acetylacetonate (400 mg) in dichloromethane (60 ml, distilled from CaH_2) under N_2 was added 3M t-butyl hydroperoxide (87 ml, 0.261 mol) in 2,2,4-trimethylpentane (87 ml, 0.261 mol) over 75 min at such a rate to keep the temperature at 25° with a water bath. After stirring 16 hr at 25° the reaction was cooled to 0° and saturated aqueous sodium sulfite (150 ml) was added over 1 hr keeping the reaction temperature below 20°. The reaction was stirred for 1.5 hr at room temperature. The layers were separated, and the aqueous layer was extracted with 50 ml of dichloromethane. The organic layers were combined, washed with water (50 ml), dried over sodium sulfate, filtered and concentrated *in vacuo*.

The residue was purified on Merck silica gel (2 l, petroleum ether: ether 1:1). The column was eluted with petroleum ether:ether (2:1) to give 24.19 g of pure 6. Fractions containing impure 6 were purified on Merck silica gel (400 ml, petroleum ether:ether 1:1). The column was eluted with petroleum ether:ether (1:1) to give 2.71 g of pure 6. Total yield of 6 was 26.90 g (84%).

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Compound 7

To a suspension of 60% sodium hydride in mineral oil (5.11 g, 0.128 mol) in tetrahydrofuran (247 ml) under N_2 was added compound 6 (25.58 g, 0.116 mol) in tetrahydrofuran (123 ml) dropwise over 20 min at room temperature. This was stirred with an overhead stirrer for 2 hr at room temperature and 1 hr at 40°. The reaction was cooled to room temperature, and benzyl bromide (15.2 ml, 0.128 mol) and tetrabutyl ammonium iodide (412 mg) were added. After 3 hr ethanol (20 ml) was added, and the reaction was stirred for 10 min. The solvents were removed *in vacuo*. The residue was partitioned between water (200 ml) and ether (200 ml). The water layer was extracted with ether (200 ml) and the organic layers were combined, dried over sodium sulfate, filtered and concentrated *in vacuo*.

The residue was purified on Merck silica gel (2 l, petroleum ether:ether 3:1). Elution with a gradient of petroleum ether:ether (3:1 to 1:1) gave 27.21 g of 7 (76% yield).

Compound 9

To a solution of 7 (6.20 g, 20 mmol) and O-benzylguanine 8 (9.64 g, 40 mmol , dried 50° *in vacuo*) in dry dimethyl formamide (80 ml, over sieves) at 60° under N_2 was added lithium hydride (80 mg, 10 mmol). The temperature was raised to 125° and stirred for 10 hr and the lowered to room temperature and stirred for 6 hr. Acetic acid (572 µl, 10 mmol) was added and the reaction was stirred for 10 min. The solvents were removed *in vacuo*, and the residue was purified on Merck silica gel (2 l, dichloromethane). The column was eluted with a gradient of dichloromethane to dichloromethane:methanol (95:5) to give 9.03 g of impure 9. This was purified on SilicAR CC-7 (1 l, chloroform) and eluted with a gradient of chloroform to chloroform:ethanol (88:12) to give 6.63 g (60% yield) of 9.

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Compound 10

To a solution of compound 9 (5.45 g, 9.89 mmol) in dichloromethane (75 ml, distilled from CaH_2) under N_2 was added p-anisylchlorodiphenylmethane (3.37 g, 10.93 mmol), triethylamine (2.35 ml, 16.81 mmol) and dimethylaminopyridine (40 mg). The reaction was stirred at room temperature for 3 hr and was then extracted with 5% sodium bicarbonate (30 ml) and water (10 ml). The organic layer was dried, filtered and concentrated *in vacuo*. The residue was purified on SilicAR CC-7 (600 ml packed in chloroform). The column was eluted with chloroform:ethanol (99:1) to give 1.5 g of pure 10. Fractions containing impure 10 were purified on SilicAR CC-7 (700 ml packed in chloroform). The column was eluted with chloroform:ethanol (99.5:0.5) to give 4.54 g of 10. Total yield of 10 was 6.04 g (74%).

Compound 11

To a solution of 10 (4.10 g , 4.88 mmol, dried by concentration from toluene) in dimethyl sulfoxide (12 ml, dried over sieves) was added dicyclohexylcarbodiimide (3.08 g, 14.9 mmol) and methyl phosphonic acid (0.239 g, 2.49 mmol). The reaction was stirred 4 hr at room temperature and then sat for 16 hr at -20°. The reaction was then warmed to room temperature and oxalic acid dihydrate (60 mg) in methanol (8.0 ml) was added. This was stirred for 2.5 hr. The reaction was filtered, and the filtrate was dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 ml), filtered and concentrated *in vacuo*. The NMR spectra indicated there was unreacted dicyclohexylcarbodiimide in the residue.

The residue was dissolved in dimethylsulfoxide (9 ml) and then methyl phosphonic acid (150 mg) in methanol (6 ml) and oxalic acid dihydrate (60 mg) were added. This was stirred for 4 hrs. The reaction was filtered and the precipitate was washed with dichloromethane (120 ml). The organic layer was washed with water (3 x 50 ml), dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 ml) filtered and concentrated *in vacuo* to give 3.73 g of 11. The NMR spectra indicated there was no unreacted dicyclohexylcarbodiimide in the residue.

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Compound 12

To a solution of 11 (1.8 g, 2.19 mmol) in dichloromethane (40 ml distilled from CaH_2) was added a slurry of 0.3M $Zn\text{-}TiCl_4\text{-}CH_2Br_2$ (40 ml, 12.3 mmol) by teflon cannula under N_2. After 3 hrs the reaction was poured slowly into saturated sodium bicarbonate (200 ml) and dichloromethane (200 ml). The mixture was filtered through celite and the celite pad was washed with dichloromethane (3 x 75 ml). The organic layers were combined, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 ml) and refiltered through celite. The pad was washed with dichloromethane (30 ml). The organic washes were combined and concentrated to give 1.43 g of 12.

Compound 13

To a solution of 12 (2.5 g, crude) in tetrahydrofuran (25 ml) and methanol (25 ml) was added 3N HCl (12.5 ml). The reaction was heated at 50° for 2.5 hr and then cooled to room temperature. The pH of the reaction was raised to 7.3 with 1N KOH, and the mixture was extracted with ethyl acetate (3 x 120 ml). The extracts were combined, dried over sodium sulfate, filtered and concentrated *in vacuo*.

The residue was purified on Merck silica gel (340 ml, packed in chloroform: ethanol, 97:3). The column was eluted with a gradient of chloroform:ethanol (97:3 to 80:20) to give 316 mg (23% yield for 3 steps) of compound 13.

Compound 14

To a solution of 13 (304 mg, 0.673 mmol) in dichloromethane (12 ml, distilled from CaH_2) at -78° under N_2 was added 1M boron trichloride in dichloromethane (6.7 ml, 6.7 mmol). The reaction was stirred at -78° for 2 hr and -40° for 30 min. It was then cooled to -78° and methanol (60 ml) was added slowly over 10 min. The reaction mixture was concentrated from methanol (4 x 40 ml). After dissolving the reaction in methanol (5 ml) and water (5 ml), the pH was adjusted to 6.8 with 1N KOH. The slurry was concentrated *in vacuo*, suspended in water and purified on CHP-20P (16 ml, water). The column was eluted with a gradient of water to water:acetonitrile (93:7) to give 115 mg (62% yield) of 14 as a solid, m.p. >220°.

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Analysis: Calc'd for $C_{12}H_{15}N_5O_3 \cdot 0.9\ H_2O$

C, 49.12; H, 5.77; N, 23.87

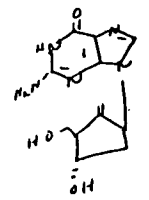
Found: C, 49.17; H, 5.87; N, 23.81

^1H NMR (270 MHz, $DMSOd_6$) δ10.52(s, 1H, NH), 7.64(s, 1H, H-8), 6.38(s, 2H, NH_2), 5.35(m, 1H, H-1'), 5.09(m, 1H, vinylic H), 4.84 (d, 1H, CHOH), 4.79 (t, 1H, CH_2OH), 4.56(m, 1H, vinylic H), 4.22(m, 1H, CHOH), 3.53(m, 2H, CH_2OH), 2.49 (m, $DMSOd_6$ and $CHCH_2OH$), 2.21(m, 1H, $CHCH_2CH$), 1.67(m, 1H, $CHCH_2CH$).

SQ 34,676

m/L030795-32
09:41:34
SQUIBB GX-270
1H-NMR#
DFILE PROTON
COMNT SQ34,676
SLVNT DMSO
SCANS 80

see p:616-001



PEAK	ms
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RESOL	0.5900000 Hz
RESOL	0.0021838 ppm
EXREF	2.4900000 ppm
OBS	-1907.49 Hz
ABOBS	270168.3000000 KHz
NSAIN	16
COMNT	SQ34,676

NO.	PPM	INT(I)	FREQ(Hz)	POSITION	BAR GRAPH
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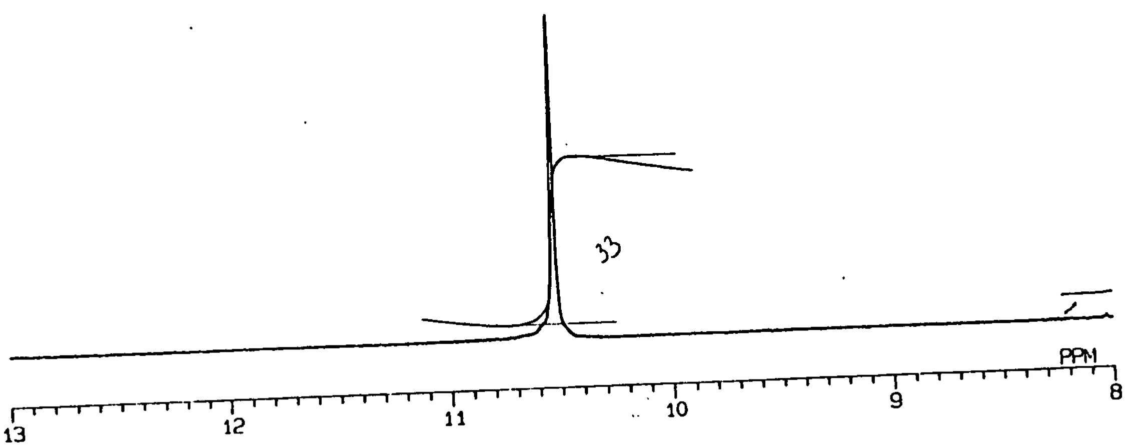
2	6.3833	7.56054	1724.56	4408	++
3	5.3850	1.23562	1454.86	4868	
4	5.3547	1.94515	1446.65	4882	
5	5.3460	1.92862	1444.30	4886	
6	5.3156	1.26635	1436.09	4900	
7	5.1007	3.44514	1378.05	4999	+
8	5.0921	4.80385	1375.70	5003	+
9	4.8447	7.57182	1308.86	5117	++
10	4.8316	6.79396	1305.35	5123	+
11	4.8121	2.96146	1300.07	5132	+
12	4.7926	7.13117	1294.79	5141	+
13	4.7730	2.86872	1289.52	5150	+
14	4.5647	3.30856	1233.23	5246	+
15	4.5360	5.66208	1230.88	5250	+
16	4.5473	3.17850	1228.54	5254	+
17	4.2240	2.79205	1141.18	5403	+
18	3.5312	4.63581	959.42	5713	+
19	3.5295	6.50401	953.56	5723	+
20	3.5056	4.52917	947.11	5734	+
21	3.3516	0.77221	905.48	5805	
22	3.2951	100.00000	890.24	5831	++++++++++++++++
23	3.2713	2.96189	883.79	5842	+
24	2.5052	4.61809	676.82	6195	+
25	2.4987	8.11704	675.06	6198	++
26	2.4922	10.38539	673.30	6201	++
27	2.4857	6.88696	671.54	6204	+
28	2.4770	3.33929	669.20	6208	+
29	2.2600	0.95518	610.57	6308	
30	2.2426	1.01113	605.87	6316	
31	2.2144	1.81845	598.25	6329	
32	2.1970	1.57334	593.56	6337	
33	2.1732	1.54653	587.11	6348	
34	2.1580	1.45215	583.01	6355	
35	2.0668	1.54053	558.38	6397	
36	2.0386	1.67068	550.76	6410	
37	1.9909	1.02140	537.86	6432	
38	1.2248	0.39791	330.89	6785	

marian young

SQ 34,676

09: 39: 47
SQUIBB GX-270
1H-NMR#________
DFILE PHOTON
COMNT SQ34, 676
SLVNT DMSO
SCANS 80

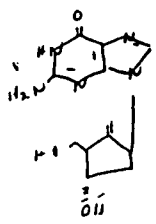


Marian Young

SQ 34 676

09: 17: 42
SQUIBB GX-270
1H-NMR#_____
DFILE PROTON
COMNT SQ34676, D2OEX
SLVNT DMSO
SCANS 80

D_2O exchan



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KOINT 604241900
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RESOL 0.0021838 ppm
EXREF 2.4900000 ppm
OBS -1906.90 Hz
ABOBS 270168.3000000 kHz
MEAIN 15
COMNT SQ34676,D2OEX

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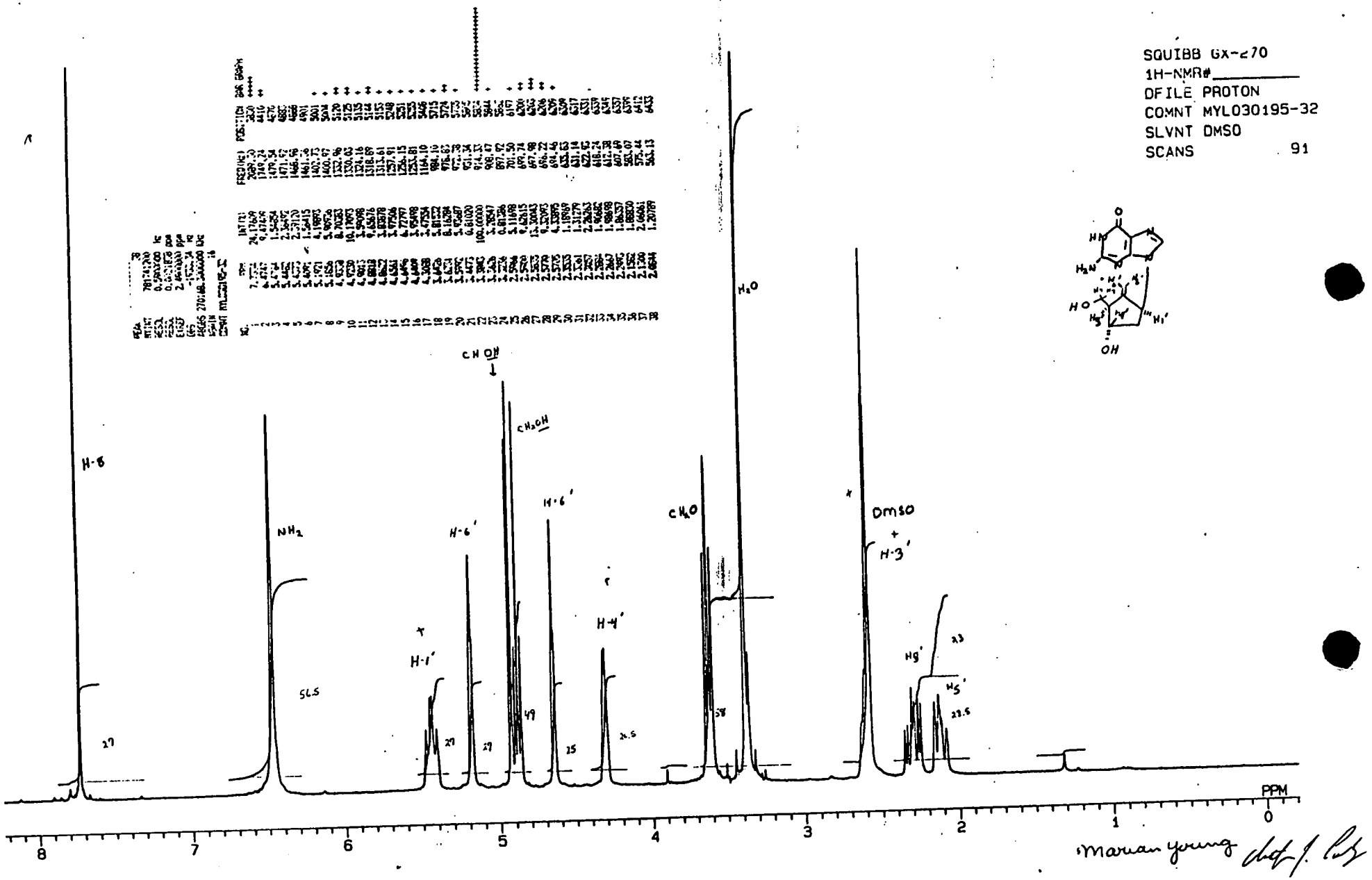
1	7.6399	28.01304	2064.03	3828	++++++
2	6.3985	1.68460	1728.66	4400	
3	5.3698	2.88856	1450.79	4874	+
4	5.3395	4.50843	1442.54	4888	+
5	5.3308	4.53215	1440.20	4892	+
6	5.3026	3.00546	1432.58	4905	+
7	5.0899	11.19917	1375.12	5003	++
8	4.9250	0.85927	1330.56	5079	
9	4.9141	0.79564	1327.63	5084	
10	4.8707	0.70015	1315.90	5104	
11	4.5473	12.75233	1228.54	5253	+++
12	4.5387	7.21691	1226.19	5257	+
13	4.2131	6.97132	1138.25	5407	+
14	4.2066	7.37009	1136.49	5410	+
15	4.1979	6.54506	1134.14	5414	+
16	3.8051	0.69842	1028.02	5595	
17	3.5295	21.60772	953.56	5722	++++
18	3.5033	24.75189	946.52	5734	++++
19	3.4688	100.00000	937.14	5750	++++++++++++++++++
20	2.5030	11.19166	676.23	6195	++
21	2.4965	18.19315	674.47	6198	+++
22	2.4900	23.73730	672.71	6201	++++
23	2.4835	16.85256	670.96	6204	+++
24	2.4770	8.05739	669.20	6207	++
25	2.2556	2.29761	609.39	6309	
26	2.2383	2.43915	604.70	6317	
27	2.2100	4.29813	597.08	6330	+
28	2.1992	3.49492	594.15	6335	+
29	2.1927	3.86253	592.39	6338	+
30	2.1688	3.61278	585.94	6349	+
31	2.1536	3.36348	581.84	6356	+
32	2.0646	3.62225	557.80	6397	+
33	2.0364	3.86318	550.17	6410	+
34	1.9887	2.26998	537.28	6432	
35	1.2074	0.79339	326.20	6792	

Marian Gyorgy


SQUIBB GX-270
1H-NMR#
DFILE PROTON
COMNT MYL030195-32
SLVNT DMSO
SCANS 91
H-8
NH2
H-1'
H-6'
CH2OH
H-6'
H-4'
CH2O
H2O
DMSO
+
H-3'
H-2'
H-5'
PPM
8
7
6
5
4
3
2
1
0

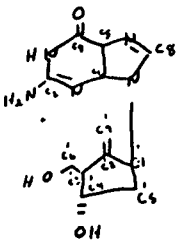
L030-195-32

SQ 34,676

MYL030195-32

SQUIBB GX-270
13C-NMR# 114778
DFILE 114778
COMNT L030-195-32
SLVNT DMSO
SCANS 10000

see p L 6 1 6 0 0 1 for write up



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NINT	771503600
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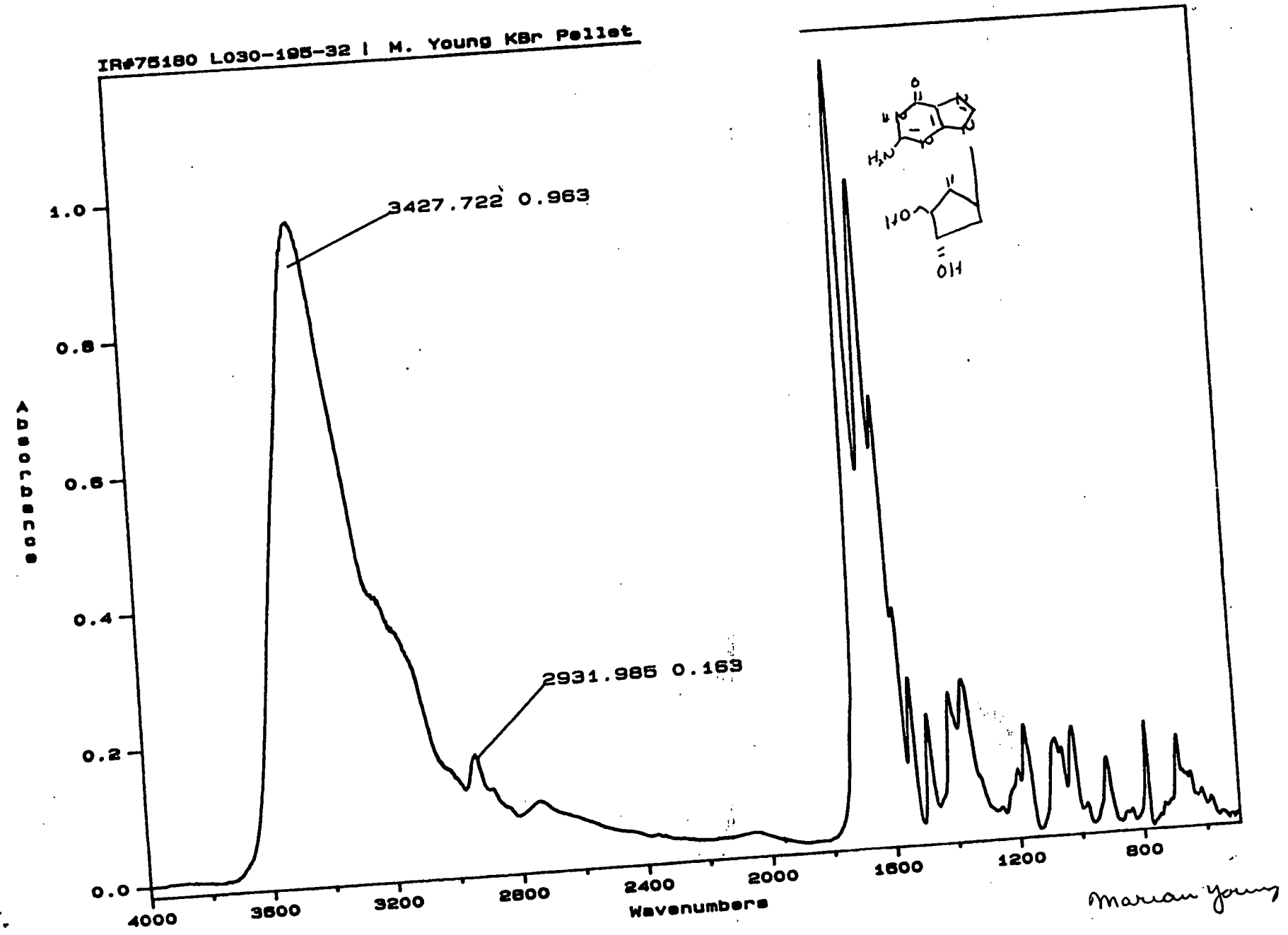
RESOL	0.0288518 ppm
EXREF	39.5000000 ppm
OBS	-7270.79 Hz
ABOBS	67940.7000000 Hz
NSAIN	20
COMNT	L030-195-32

NO.	PPM	INT(%)	FREQ(Hz)	POSITION	BAR GRAPH
1	156.760	3.17781	10649.2	2369	+
2	154.443	0.37064	10627.7	2380	
3	153.420	4.73118	10422.3	2485	+
4	151.375	3.16728	10283.1	2557	+
5	151.251	4.94753	10274.6	2561	+
6	135.882	5.49120	9231.0	3098	+
7	127.416	0.36401	8658.8	3388	
8	123.336	0.34718	8380.0	3529	
9	116.214	2.31603	7894.8	3777	
10	111.981	0.36756	7607.3	3924	
11	109.188	4.46920	7417.5	4021	+
12	73.250	-0.37168	4976.1	5269	
13	70.341	6.10648	4778.5	5370	+
14	62.969	5.47076	4277.7	5626	+
15	55.050	4.87710	3739.7	5901	+
16	54.042	6.11286	3671.3	5936	+
17	40.421	13.38528	2746.0	6409	+++
18	40.134	37.55621	2726.4	6419	+++++++
19	39.817	83.55396	2704.9	6430	+++++++++++++++
20	39.500	100.00000	2683.4	6441	+++++++++++++++++
21	39.183	81.03498	2661.9	6452	+++++++++++++++
22	38.895	39.78599	2642.3	6463	+++++++
23	38.579	14.62220	2620.8	6473	+++
24	37.974	0.52894	2579.7	6494	
25	37.542	0.41808	2550.3	6509	
26	17.010	-0.36595	1155.5	7222	
27	15.340	-0.38421	1042.1	7280	

marian
yung

SQ 34676


IR#75180 L030-195-32 | M. Young KBr Pellet
3427.722 0.963
2931.985 0.169
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
4000
3600
3200
2800
2400
2000
1600
1200
800
Wavenumbers
Marian Young

Peak Pick cm-1	Intensity
644.265	0.063
656.839	0.062
684.773	0.119
783.149	0.143
912.388	0.092
1016.550	0.143
1053.200	0.112
1072.489	0.126
1170.865	0.150
1195.941	0.085
1363.759	0.224
1408.125	0.207
1481.424	0.176
1535.434	0.232
1570.155	0.336
1597.160	0.650
1629.952	0.970
1683.963	1.149
2879.904	0.112
2931.985	0.163
3211.681	0.399
3219.397	0.404
3232.900	0.408
3427.722	0.963

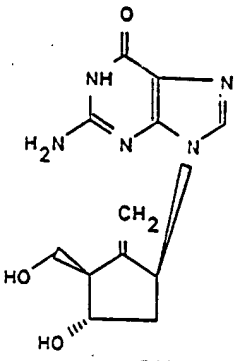
Mariam Jones

ATTACHMENT B PRELIMINARY

IN VITRO ANTIVIRAL ACTIVITY CMV

Compound: SQ 34676 FW 277.28

Structure:



Concentrations: 25 mg/1 ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μM)

ED_{50} CMV AD169: 36-36.1 *

Control (μM) SQ 31917 (DHPG)

ED_{50} CMV AD169: 2-4

Comments:

CONC μM	CONC μg/ml	% REDUCTION
361	100	100
36.1	10	57
3.61	1	36

Notebook Page: L374-077

Assay Date: ___ Analyst: A.V. Tuomari, B. McGeever-Rubin

Copies to: DR. W. SCOTT, DR. A.K. FIELD, DR. W. KOSTER, DR. B. TERRY, DR. R. ZAHLER, DR. G. BISACCHI, DR. W. SLUSARCHYK, Dr. M. Haffey, Dr J. Tino, DR. V. GOODFELLOW, DR. G. YAMANAKA, DR. G. VITE, DR. S. AHMAD

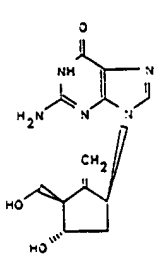
* TO BE REPEATED

ATTACHMENT C

IN VITRO ANTIVIRAL ACTIVITY

Compound: SQ 34676

Structure:



Concentrations: 20 mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 cells

Compound (μM)

ED_{50} HSV-1 (SCH): <3.6 ED_{50} HSV-2 (186): <3.6 Toxicity: NT 361

Control (μM) 31933 (ACV)

ED_{50} HSV-1 (SCH): 0.22-0.44 ED_{50} HSV-2 (186): 0.22

Comments:

PERCENT REDUCTION OF PLAQUES

ug/ml	uM	SCH	186
100	361	100 %	100 %
10	36	100 %	100 %
1	3.6	73 %	55 %

Notebook Page: L300 ; 063-065

Assay Date: _____ Analyst: B McGeever / AV Tuomari

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DR. G. YAMANAKA
DR. G. VITE
Dr. S. Ahmad

* preliminary results

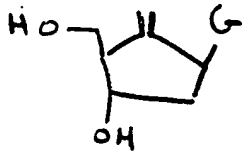
ATTACHMENT D

CMV

IN VITRO ANTIVIRAL ACTIVITY

Compound: SQ 34676

Structure:



Concentrations: 50 mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (uM)

ED_{50} CMV (AD 169): 90

Control (uM) SQ 31919

ED_{50} CMV (AD 169): 0.8 - 2

Comments:

CONC		% REDUCTION
uM	ug/ml	
361	100	72
180	50	65
90	25	47
36.1	10	6
18	5	19

Notebook Page: L374-098

Assay Date: ____ Analyst: A.V. Tuomari, B. McGeever-Rubin

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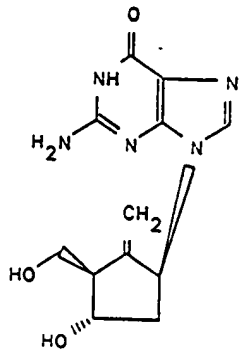
DR. G. VITE
DR. S. AHMAD

ATTACHMENT E

VZV IN VITRO ANTIVIRAL ACTIVITY (ELLEN)

Compound: SQ 34676 FW. 277.28 Structure:



Concentrations: 50 mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μM)

$ED_{50}^{VZV}_{ELLEN}$: ≤ 7.2

Control (μM) SQ 31933

$ED_{50}^{VZV}_{ELLEN}$ 2-4

Comments:

CONC.		% REDUCTION
μM	μg/ml	
361	100	100
180	50	100
90	25	92
36.1	10	87
18	5	69
7.2	2	56

Notebook Page: L374-097

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DR G. VITE
DR. S. AHMAD

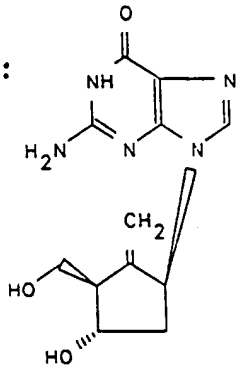
Assay Date: ____ Analyst: A.V. Tuomari, B. McGeever-Rubin

ATTACHMENT F

IN VITRO ANTIVIRAL ACTIVITY VZV ELLEN

Compound: SQ 34676

Structure:



Concentrations: 50 mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μM)

ED_{50} VZV ELLEN: 19-36

Control (μM) SQ 31933

ED_{50} VZV ELLEN: 2-4

Comments:

CONC. μM	CONC. μg/ml	% REDUCTION
94	25	86
36.1	10	65
18.8	5	30
7.5	2	10

Notebook Page: L374-105

Assay Date:

Analyst: B. McGeever Rubin
A.V. Tuomari

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DR. S. AHMAD

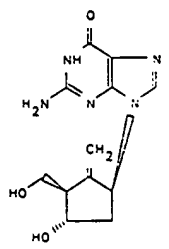
ATTACHMENT 6

* VZV ppIIA

IN VITRO ANTIVIRAL ACTIVITY

Compound: SQ 34676

Structure:



Concentrations: 20 mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION in WI-38 cells

Compound (μM)

ED_{50}: VZV ppIIA: 43.6

Control (μM) 31933 (ACV)

ED_{50} VZV ppIIA: 0.22-0.44

Comments:

μg/ml	μM	% Reduction
100	361	100 %
10	36	100 %
1	3.6	87 %

Notebook Page: L300; 063-065

Assay Date: ___ ___ Analyst: BMcGeever AVTuomari

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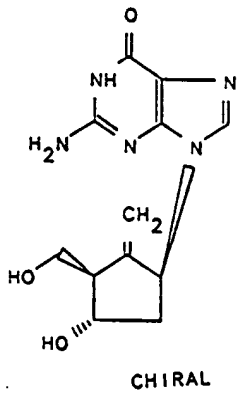
Dr. M. Haffey
Dr J. Tino
DR. V. GOODFELLOW
DR. G. YAMANAKA
DR. G. VITE
DR. S. AHMAD

* VZV (ppIIA) - wildtype VZV strain
Sawyer et al., 1988

ATTACHMENT H

IN VITRO ANTIVIRAL ACTIVITY HSV

Compound: SQ 34676 FW 277.28 Structure:



CHIRAL

Concentrations: 50mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μM)

ED_{50} HSV-1 (SCH): 3.6 ED_{50} HSV-2 (186): 7.2 - 18 Toxicity: NT at 9C

Control (μM) SQ 31933 (ACV)

ED_{50} HSV-1 (SCH): 0.2 ED_{50} HSV-2 (186): 0.2

Comments:

PERCENT REDUCTION OF PLAQUES

CONC μM	CONC μg/ml	SCH	186
90	25	—	92
36	10	99	82
18	5	97	60
7.2	2	78	31
3.6	1	51	10
1.8	0.5	24	14
0.72	0.2	13	—

Notebook Page: L374-078

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DR. W. SLUSARCHYK
DR. J. TINO
DR. M. HAFFEY
DR. V. GOODFELLOW
DR. G. YAMANAKA
DR. G. VITE

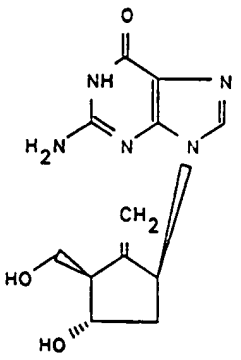
Assay Date: Analyst: A.V. Tuomari

ATTACHMENT I

CELL GROWTH INHIBITION

CHIRAL

Compound: SQ 34676 Structure:



Solvent: DMSO

Assay Procedure: Inhibition of WI-38 cell proliferation after 3 days in the presence of compound

Results (μM): ED_{50} 450

CELL GROWTH INHIBITION AS PERCENT OF CONTROL

Conc. μM	%
600	50.0
150	37.5
38	72.9
10	68.0

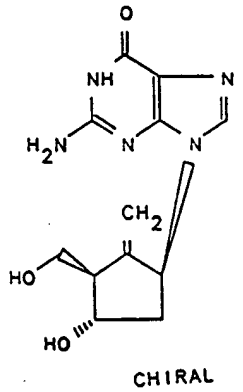
Notebook Page: L799 c 013
Assay Date:
Analyst: P. Vetter

Copies to: Dr. A. K. Field
Dr. M. L. Haffey
Dr. R. Zahler

ATTACHMENT J

CELL GROWTH INHIBITION

Compound: SQ 34676 NN001 Structure:



CHIRAL

Solvent: DMSO

Assay Procedure: Inhibition of WI-38 cell proliferation after 3 days in the presence of compound

Results (μM): ED_{50} 310

CELL GROWTH INHIBITION AS PERCENT OF CONTROL
(3 DAYS POST-COMPOUND)

Conc. μM	%
800	17
200	32
50	66
12.5	106

Notebook Page: L374-170
Assay Date:
Analyst: A.V. Tuomari

Copies to: Dr. A. K. Field
Dr. M. L. Haffey
Dr. R. Zahler